REMARKS

I. Claim Amendments

Claim 1 has been amended by limiting the age of the adult avian from which the testis is obtained to one that is aged 2-70 weeks. Support for this amendment can be found throughout the specification, in particular, at page 5, lines 17-21 of the specification.

In addition, claims 1, 20, and 25 have been amended so that the long-term culture of spermatogonial stem cells is carried out for more than 3 months. Support for this amendment can be found at page 34, lines 9-12 of the specification.

Further, step (c) of claims 1, 20, and 25 has been separated into two more specific steps, (c) and (d), where new step (c) includes culturing the population of testicular cells for about 5 to 10 days on plates in order to obtain a colony of spermatogonial stem cells, and step (d) encompasses the steps of taking the colony of spermatogonial stem cells and culturing the cells for more than about 80 to 85 days. Support for the newly amended steps (c) and (d) can be found at page 33, line 27 through page 34, line 16 of the present specification.

Furthermore, claims 1, 20, and 25 have been also amended so that step (b) is carried out by treating the avian testis with a mixture of collagenase and trypsin; the medium of steps (c) and (d) includes FBS, avian serum, non-essential amino acids, Hepes buffer, and β-mercaptoethanol; and the feeder cell is an avian gonadal stroma cell or avian testicular stroma cell. Support for these amendments can be found throughout the present specification. In particular, page 19, line 21 through page 20, line 8 of the present specification discloses the medium added with the above-noted supplements.

Claims 2-6 have been cancelled.

In addition, typographical errors were corrected in claims 7, 15, and 17. In particular, the term "glia-derived neurotrophic factor" has been replaced with "glial-derived neurotrophic factor," the term "a6-integrin antibody" has been replaced with "α6-integrin antibody," and the term "pl-integrin antibody" has been changed with "β1-integrin antibody".

No new matter has been added.

The preceding amendments and the following remarks are believed to be fully responsive to the outstanding Office Action and are believed to place the application in condition for allowance.

The Examiner is respectfully requested to reconsider and withdraw the rejections in view of the amendments and remarks as set forth below.

II. Rejection under 35 U.S.C. § 103

The Examiner has rejected claims 1-15 and 20-25 under 35 U.S.C. § 103 (a) as being unpatentable over Baguisi et al. (U.S. Patent Application Publication No. 2002/0162134), in view of Shinohara et al. (PNAS 97:8346-8351; 2000) and, in the case of claims 1, 3, and 11, further in view of Shinohara et al. (U.S. Patent Application Publication No. 2006/0265774).

As noted in the claim amendment, claims 1, 20, and 25 have been amended so that they are directed to methods for long-term culture of avian spermatogonial stem cells for more than 3 months. As is shown in the panel (d) of figure 9 of the present specification, chicken spermatogonial stem cells have been cultured successfully by the method of the present invention.

In addition, as noted in the newly revised steps (c) and (d) of claims 1, 20, and 25, the

isolated testicular cell population is cultured on plates as a primary culture for about 5 to 10 days to form a colony of spermatogonial stem cells, and then the spermatogonial stem cells of the colony obtained from primary culture are further cultured on a feeder cell layer for more than about 80-85 days.

It is noteworthy that the medium used for culturing the avian SSCs (spermatogonial stem cells) in steps (c) and (d) of claim 1 includes FBS (fetal bovine serum), avian serum, non-essential amino acids, Hepes buffer, and β-mercaptoethanol. This medium supplemented with the above factors is described as "DMEM-C" in the present specification.

As described in the present specification, page 28, line 1 through page 29, line 1, where avian SSCs are cultured in the medium "DMEM-C," the number of colonies of avian SSCs was increased about **14-fold** as compared to that cultured in DMEM-B, the basic DMEM medium only supplemented with FBS.

Although Baguisi et al. discloses DMEM medium supplemented with 10% FBS and 5% chicken serum, and Shinohara et al. also discloses that culture medium for testes cells supplemented with fetal calf serum and 2-mercaptoethanol, the specific medium containing FBS (fetal bovine serum), avian serum, non-essential amino acids, Hepes buffer, and β-mercaptoethanol is not disclosed or suggested in the cited references.

Further, as described in the present specification, page 28, lines 7-10, such higher population of avian SSCs are attributed to the combination of supplements of chicken serum, non-essential amino acids, Hepes buffer, and β-mercaptoethanol.

The employment of DMEM-C media in the present method makes it possible to culture avian SSCs for long-term more than 3-months.

Claims 1, 20, and 25 of the present invention are further limited by the specification of treating avian testis with a mixture of collagenase and trypsin.

As described in the results in Table 1 and page 24, lines 5-20 of the present specification, among the methods for isolation of testicular cells from the avian testis the method ("method 3") using a mixture of collagenase and trypsin exhibited the highest cell viability. Moreover, method 3 could be carried out in a more convenient and shorter time manner than the other two methods.

Although Shinohara et al. also disclose that the testis of a just born mouse is totally digested with collagenase, trypsin, and DNase, they did suggest neither using the mixture of collagenase and trypsin, nor the effectiveness of them.

Claims 1, 20, and 25 of the instant invention are also amended by limiting the feeder cells to avian gonadal stromal cell or avian testicular stroma cell. According to the results of the present experiments shown in Fig. 2 and at page 26, line 21 through page 27, line 15, where the avian SSCs are co-cultured with avian gonadal stroma cell (GSC) or testicular stroma cell (TSC), the number of SSCs is higher than that obtained with co-culture with chicken embryonic fibroblasts (CEF) or mouse STO cells (STO).

Although Baguisi et al. also disclose PGCs co-cultured with the gonadal stromal cells, they did not suggest the superiority of using the avian gonadal stroma cells or avian testicular stroma cells as feeder cells in culturing the avian SSCs.

In summary, the technical feature of the presently amended claims 1, 20, and 25 lies in the long-term culture of avian spermatogonial stem cells for more than 3 months. The long-term culture of avian spermatogonial stem cells for more than 3 months is not described or suggested in the cited references of Baguisi et al. and Shinohara et al.

The method of long-term culture of avian SSCs can be used to prepare more reliable SSCs populations and to provide helpful tools for understanding the principles underlying spermatogenesis. In addition, avian SSCs of this invention are useful in producing transgenic aves by gene manipulation.

The references of Baguisi et al. and Shinohara et al. do not explicitly or implicitly suggest any desirability or advantage of the technical features of the present invention claimed in claims 1, 20, and 25. Therefore, it would not have been obvious for a person of ordinary skill in the art to carry out the invention of claims 1, 20, and 25 of the present invention.

Consequently, Applicants respectfully request that this rejection be reconsidered and withdrawn.

CONCLUSION

With respect to the rejection under 35 U.S.C. § 103, since the references of Baguisi et al., Shinohara et al. (PNAS), and Shinohara et al. (US 2006/0265774) do not suggest the desirability, advantage, or motivation to arrive at the technical features of the present inventions of claims 1, 20, and 25, it would not have been obvious for a person of ordinary skill in the art to carry out the invention of claims 1, 20, and 25 of the present invention.

Therefore, in view of the foregoing remarks, Applicants respectfully request reconsideration and timely allowance of the pending claims.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Susan M. Michaud

Susan M. Michaud, Ph.D.
Reg. No. 42,885

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045